UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/032,972	02/26/1998	ACHIM H. KROTZ	ISIS-2710	1518

7590 02/04/2003

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
ONE LIBERTY PLACE 46TH FLOOR
PHILADELPHIA, PA 19103

EXAMINER	
CRANE, LAWRENCE E	
ART UNIT	PAPER NUMBER
1623	

DATE MAILED: 02/04/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/032,972	Applicant(s) Krotz et al.	
	Examiner L. E. Crane	Group Art Unit 1623	

- THE MAILING DATE of this communication appears on the cover sheet beneath the correspondence address -

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE --3-- MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be filed after six months from the date of this communication.
- If the prior for reply specified above is less that thirty (30) days, a reply within the statutory minimum of thirty days will be considered timely.
- If NO period for reply is specified above, such period shall ,by default, expire SIX (6) MONTHS from the date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 USC §133).

Status

[X] Responsive to communication(s) filed on -12/02/02 (amdt F)-.
[X] This action is **FINAL.**
[] Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle,* 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

[X] Claims ---1-42--- are pending in the application. Claims -[]- have been cancelled.
Of the above claim(s) ---[]--- is/are withdrawn from consideration.
[] Claim(s) ---[]--- is/are allowed.
[X] Claims ---1-42--- are rejected.
[] Claim(s) ---[]--- is/are objected to.
[] Claim(s) ---[]--- are subject to restriction or election requirement.

Application Papers

[] See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
[] The proposed drawing correction, filed on -[]- are [] approved [] disapproved.
[] The drawing(s) filed on -[]- is/are objected to by the Examiner.
[] The specification is objected to by the Examiner.
[] The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119(a)-(d)

[] Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119 (a)-(d).
[] All [] Some* [] None of the CERTIFIED copies of the priority documents have been
[] received.
[] received in Application No. (Series Code/Serial Number) -[]-.
[] received in the national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: -[]-.

Attachment(s)

[] Information Disclosure Statement(s), PTO-1449, Paper No(s). --[]--
[X] Notice of Reference(s) Cited, PTO-892
[] Notice of Draftsperson's Patent Drawing Review, PTO-948
[] Interview Summary, PTO-413
[] Notice of Informal Patent Application, PTO-152
[X] Other: -[]- Ref Attached to P.M. 25.

No claims have been cancelled, claims **1, 21 and 42** have been amended, and no new claims have been added as per the amendment filed December 2, 2002. An Associate Power of Attorney has also been received on December 2, 2002 and made of record.

Claims **1-42** remain in the case.

The following is a quotation of 35 U.S.C. §103(**a**) which forms the basis for all obviousness rejections set forth in this Office action:

> "A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made."

Claims **1-42** are rejected under 35 U.S.C. §103(**a**) as being unpatentable over **Ravikumar '621** (PTO-892 ref. **A**) in view of **Caruthers et al. '679** (PTO-892 ref. **G**) and further in view of **Froehler et al. '076** (PTO-892 ref. **H**) and further in view of **Sproat et al.(I)** (PTO-892 ref. **W**), **Conway et al.** (PTO-892 ref. **Y**), **Atkinson et al.** (PTO-892 ref. **Z**), and **Sproat et al.(II)** (PTO-892 ref. **RA**).

The instant claims are directed to entirely conventional, 7 step oligonucleotide syntheses conducted using an automated device to execute steps 2-6 {aka steps b) through f)}, wherein the two variations from the prior art are i) the choice of solvent or solvent mixture present for deprotection step (c) and ii) the choice of substrate as a linear oligonucleotide as opposed to the branched oligonucleotide of the prior art.

Ravikumar '621 (PTO-892 ref. **A**) discloses entirely conventional oligonucleotide synthesis wherein the solvent for the coupling step is acetonitrile in the examples and the P-protecting group varies from the conventional phosphorus-ester protecting group. At column 3 this reference refers to several different patents which disclose the solid phase synthesis of oligonucleotides including three Caruthers et al. patents now cited herein as PTO-892 references **I, J and K**. Each of these Caruthers et al. patents discloses the automation of the synthesis of oligonucleotides via process steps closely analogous to, if not identical with, the process steps claimed herein, the most detailed disclosure occurring in Caruthers et al. '418 (PTO-892 ref. **K**). In the **Ravikumar '621** patent at column 10, lines 1-16, a generic disclosure of the process steps leading to an oligonucleotide is presented, including acid-mediated deprotection of the 5'-hydroxyl moiety of a solid-support-attached nucleoside. However, no disclosure of any preferred solvent for the required acid reagent is included. In the same column at line 50, the removal of 5'-hydroxyl protection by contact with acid from a solid-support-attached oligonucleotide is also taught without specifying any particular solvent. At column 14, lines 5-28, a more complete disclosure of possible 5'-hydroxyl protecting groups is provides along with a list of acids effect to deprotect, but no preferred solvents are listed. At column 18, lines 37-41, deprotection is accomplished by contact with a solution of dichloroacetic acid in dichloromethane, conditions repeated in subsequent experimental procedures. The choice of any particular deprotection solvent is therefore apparently a choice within the purview of the ordinary practitioner in view of this disclosure. This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Caruthers et al. '679 (PTO-892 ref. **G**) at column 5, lines 10-14, teaches the use of "... any solvent which will dissolve the reactants ..." including a list of specific organic solvents for phosphoramidite-intermediate-based oligonucleotide synthesis. The context of this statement suggests that Caruthers was making reference to the coupling step. However, the same generic teaching appears to also apply to the deprotection step where four different solvent/reagent systems were disclosed by Caruthers as effective in the 5'-O-detritylation process:
(1) see column 16, Table IV, footnote 1 ($ZnBr_2$ in nitromethane);
(2) see column 16, Table V, footnote 1 (toluenesulfonic acid in chloroform:methanol (7:3));
(3) see column 18, lines 26-28 ($ZnBr_2$ in nitromethane:methanol (19:1)); and
(4) see column 19, lines 47-50 (80% acetic acid).
This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Froehler et al. '076 (PTO-892 ref. **H**) discloses the use of H-phosphonate intermediates for the coupling step in the synthesis of oligonucleotides and phosphorothioate analogues thereof, including reference to the automated synthesis thereof using a "Biosearch Model 8600 DNA synthesizer" at column 9, lines 22-23. This reference also teaches the use of " ... an anhydrous organic solvent, preferably pyridine/acetonitrile ...," at column 5, lines 26-28. This "what ever works best" philosophy apparently also applies to the deprotection step; see column 5, lines 38-47. The last line of this portion of column 5 is particularly instructive. After listing 3 (three) different deprotection reagent/solvent mixtures, **Froehler** suggests a very flexible "whatever works" approach by further stating that "[o]ther deprotection procedures suitable for other known protecting groups will be apparent

to the ordinary practitioner." This reference does not disclose the particular mixture of solvents selected for use in the instant claimed processes.

Sproat et al. (I) (PTO-892 ref. **W**) discloses at p. 52, (lines 2 and 18) that toluene is useful for the purification of synthetic nucleoside intermediates. Additionally, this reference discloses at pp. 64 (Protocol 17, step 3) and page 70 (Protocol 25, step 4) that benzene is a solvent for key oligonucleotide synthesis reagents and for nucleoside-3'-O-phosphoramidites, and may be used to co-evaporate triethylamine therefrom.

Conway et al. (PTO-892 ref. **Y**) is directed to the chemical synthesis of labeled DNA and at p. 218, Section C, Subsection 2, discloses the specific use of toluene as an effective solvent for dissolution of pyridine-contaminated dinucleoside monophosphorothioate d[Cp(s)C] prior to co-evaporative removal of the pyridine/toluene mixture therefrom. The instant reference does not disclose that toluene is used in the coupling step required to make this compound.

Atkinson et al. (PTO-892 ref. **Z**) discloses at p. 43 in section (xvii), that toluene is useful to dissolve the 3'-O-phosphoramidites of 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyuridine as the first step in a re-precipitation or recrystallization process. This reference also teaches at p. 76, section 7.5, "Variation in Procedures," although no specific teaching of the substitution of an aromatic solvent from other solvents used in oligonucleotide synthesis is present in this section. In section 8.7 at p. 80, "toluene" is listed as a reagent useful in the preparation of "Deoxyribonucleoside-derivatized supports." This reference at the noted locations does not disclose the particular set of

solvents claimed herein as useful in the coupling step of an oligonucleotide synthesis.

Sproat et al.(II) (PTO-892 ref. **RA**).at p. 84, lines 10 and 9 from the end of the page, discloses that the "[p]urity of solvents and reagents is of the utmost importance as far as reliability and reproducibility of the [oligonucleotide synthetic] method are concerned." This reference also discloses at p. 93, section (xv), that a di-protected adenosine derivative may be effectively dissolved in toluene prior to evaporative solvent removal for the purpose of co-evaporating residues of pyridine therefrom (see also p. 96, section (vi) for a similar disclosure). Additionally, at p. 111, section 7.6, the listing of solvents useful in oligonucleotide synthesis includes both benzene and toluene. This reference at the noted locations does not disclose the particular set of solvents claimed herein as useful in the coupling step of an oligonucleotide synthesis.

The teachings of the prior art **Caruthers '679** and **Froehler '076** references motivate the selection of practically any organic solvent or solvent mixtures which will dissolve the reactants and not otherwise interfere with the intended synthetic transformation. The first three references (**A, G and H**) and the additional **Caruthers et al.** patents cited by **Ravikumar et al. '621** provide descriptions of conventional prior art processes for making oligonucleotides via phosphoramidite or H-phosphonate intermediates, including the 5'-O-deprotection process step and including details of how the process has been automated in reference **H** and by patents cited in reference **A**. The noted portions of the **Caruthers '679** and **Froehler '076** both teach that the choice of a particular solvent or solvent mixture is a variable clearly within the purview of the ordinary practitioner. The **Sproat et al. (I) (W)**, **Conway et al.**, **Atkinson et al.**, and **Sproat et al. (II)(RA)**

references are each generally directed to oligonucleotide synthesis thereby providing proper motivation to combine with the primary references. The secondary references provide disclosures that at least two different nucleoside-3'-O-phosphoramidites, at least one dinucleotide derivative, and some other nucleoside derivatives may be effectively dissolved in the aromatic hydrocarbon solvents benzene and/or toluene. These disclosures are deemed to provide factually specific motivations for the ordinary practitioner conducting routine experimentation to substitute toluene, benzene, or their closely related aromatic solvent relatives as substitutes for at least a portion of the solvents typically used during the deprotection step in oligonucleotide synthesis. And lastly, in light of the absence of any unexpected results, the choice of substrate (linear vs. branched oligonucleotide) is deemed to not be a basis for finding patentable distinction over the prior art of record. For these reasons the instant process claims are deemed to be lacking in any patentable distinction in view of the noted prior art.

Therefore, the instant claimed oligonucleotide processes would have been obvious to one of ordinary skill in the art having the above cited references before him at the time the invention was made.

Applicant's arguments filed December 2, 2002 have been fully considered but they are not persuasive.

Applicant argues that the prior art has been misinterpreted by examiner, and that the interpretation of the primary references is not as presented in the instant rejection. In particular, applicant argues that references **A**, **G**, and **H** do not teach that solvent choice in detritylation is critical. Applicant argues that the silence of reference "**A**" concerning the solvent in the deprotection step is "intended to convey only that the Ravikumar invention will work with any [detritylation step] solvent *that*

is suitable, but does not say anything about the scope of solvents that *are* suitable." (emphasis in original) Examiner sees this statement by applicant as a concurrence, namely that applicant agrees that the ordinary practitioner is free to determine the scope of what solvent, solvents or mixture of solvents is "suitable," or in other words, that routine experimentation is not limited.

With regard to the Caruthers '679 reference (**G**), applicant argues that because "[deprotection] rates are more variable with protic acids," applicant concludes that " ... the Caruthers reference teaches away from such variability in deprotection rate." Examiner respectfully disagrees. Applicant is quoting Caruthers '679 in a manner which ignors the reason for the preference, namely that consistent and/or predictable reaction rates made it easier to apply the then available laboratory automation to oligonucleotide synthesis. Modern commercially available oligonucleotide synthesis machines are substantially more sophisticated, and consequentially more flexible, and therefore are not so limiting to the ordinary practitioner seeking to research the optimal conditions for any given oligonucleotide synthesis. Examiner therefore concludes that this portion of applicant's argument lacks sufficient weight to overcome examiner's original analysis of the teachings of the Caruthers '679 reference.

Applicant subsequently argues that the disclosure of detritylation of an oligonucleotide using 80% acetic acid for one hour occurring at column 19 of Caruthers '679 is not applicable because the oligonucleotide in question had already been separated from its solid support. Examiner respectfully disagrees, and questions what the presence or absence of attachment to a solid support has to do with the possible application of any given reagent to effect a chemical reaction with a functional group on an oligonucleotide? The ordinary

practitioner is not walking around with blinders on, and will seek out appropriate chemical reagents from any available source so long as the reagents are being used to effect the desired transformation. For example 80% acetic acid would probably be a better choice as a detritylation reagent than a stronger acid because the lower pK_a of acetic acid may mean a lower rate of acid catalyzed depurination. Examiner therefore disagrees with applicant's very narrow interpretation of what constitutes a proper source of useful chemical knowledge which may be applied by the ordinary practitioner seeking to optimize a chemical process.

Applicant also argues that the **Froehler '076** reference (**H**) is misinterpreted by examiner to the effect that an "anything that works" philosophy applies to the choice of solvents and reagents to effect deprotection/detritylation. Applicant points out that Froehler states that "[o]ther deprotection procedures suitable for other known protecting groups will be apparent to the ordinary practitioner," and argues that the "other known protecting groups" and the "procedures suitable for their removal" is not a license to make solvent substitution on a routine basis. Examiner respectfully disagrees, and notes again that modern oligonucleotide synthesis machines are no longer so rigidly limiting as were those which Caruthers and Froehler were constrained by, and that routine experimentation is <u>not</u> either scientifically or legally prohibited because the older or any newer variety of synthesis machine is in use. To assert otherwise is to fly in the face of the experience of practitioners of modern chemical science, wherein many discoveries have occurred during routine experimentation and research; e.g the discovery of teflon as a coating inside polyethylene bottles used to store fluorine.

Examiner's disagreement with applicant exceptionally narrow analysis of the primary references also applies to the secondary references which applicant dismisses on the basis that ht primary reference do not teach what examiner has pointed out they certainly do teach, namely that the ordinary practitioner has wide latitude to substitute solvents in the course of routine experimentation.

Applicant's last argument is that the instant rejection is predicated on an "obvious to try" standard. Examiner respectfully disagrees. The motivation is present in all of the primary references and is, contrary to applicant's view, that the ordinary practitioner may elect to substitute solvents and reagents in the deprotection process steps freely without regard to structure, so long as the reagent and/or solvent does not interfere with the process step being conducted. Because the motivation is not narrowly stated does not mean the motivation is absent.

And lastly, applicant has failed to provide any showing of unexpected results which would support a decision distinguishing the instant claimed process from the prior art cited in this, or the subsequent, rejection.

Claims **1-42** are rejected under 35 U.S.C. §103(**a**) as being unpatentable over **Horn et al.** (PTO-892 ref. **WA**; previously cited as PTO-1449 ref. **CB**) in view of **Horn et al.** (PTO-892 ref. **UA**).

The subject matter of the instant claims is described in the previous rejection.

Horn et al. (WA) at page 6965, first complete paragraph (lines 19-28), discloses the use of dichloroacetic acid in toluene for the trityl deprotection step in the synthesis of branched oligonucleotides. Horn

notes in particular that a higher than usual (for single deprotection) concentration of dichloroacetic acid effects rapid de-tritylation when multiple de-tritylations must be conducted simultaneously in the parallel extensions of separate oligonucleotide chains is required for the synthesis of multiply branched oligonucleotide "fork and comb" type probes.

Horn et al. (UA) at page 4844, columns 1-2 (following the header "**Oligonucleotide synthesis**"), discloses further details relevant to the application of a mixture including dichloroacetic acid and toluene/methylenechloride to effect the de-tritylation of linear 5'-tritylated oligonucleotide precursors during the process of oligonucleotide chain extension. See particularly page 4844, column 2 at lines 6-8 and 25-28.

The prior disclosures of standard phosphoramidite-type oligonucleotide syntheses of either branched or linear oligonucleotides wherein the de-tritylation step relies on a mixture comprising dichloroacetic acid and toluene are deemed to be teachings which individually, or in combination, read on the instant claimed process. For this reasons the instant process claims are deemed to be lacking in any patentable distinction in view of the cited prior art.

Therefore, the instant claimed oligonucleotide processes would have been obvious to one of ordinary skill in the art having the above cited references before him at the time the invention was made.

Applicant's arguments filed December 2, 2002 have been fully considered but they are not persuasive.

Applicant asserts that the Horn {formerly **CB**, now **WA**} reference " teaches 1) that dichloroacetic acid in methylene chloride is standard;

and that 2) the toluene solution was needed for deprotection of branched DNA ... ." Applicant then argues that "those of skill in the art would not be lead to use the stringent deprotection regime disclosed in the Horn ... reference for standard synthesis of linear oligonucleotides." Examiner respectfully disagrees, and notes that applicant has failed to provide a reason for jumping to the stated conclusion concerning the view of Horn **WA** as prior art by the ordinary practitioner. From Examiner's perspective, Horn **WA** provides a teaching that dichloroacetic acid is effective in hydrolytic detritylations in either methylene chloride solution or in toluene, but that the latter alternative causes a faster detritylation. Examiner concludes that the ordinary practitioner seeking to optimize the efficiency of <u>any</u> oligonucleotide synthesis process which includes a detritylation step would be motivated to substitute the Horn **WA** deprotection step variation without worrying about the circumstances which lead Horn to try this variation.

Applicant then argues that the synthesis of the linear portion of the ultimate products (branched DNA's) in Horn **UA** does not read on the instant process because the sequence was not cleaved from the solid support. Examiner respectfully disagrees, and directs applicant's attention to Horn **UA** at page 4844, column 2, lines 20-22, wherein Horn discloses that a "portion of the CPG support was removed for HPCE analysis." Examiner also directs applicant to page 4846 wherein the HPCE at Figure 2A clearly illustrates that the noted linear-oligonucleotide-modified CPG sample must have been subject to cleavage of its attached oligonucleotides to produce the data presented. Cleavage conditions for the ultimate branched DNA are disclosed at page 4844 at lines 40-42, and are typical for cleavage of solid-support-attached ODN's from CPG, leading examiner to conclude that the same or very similar

conditions where applied prior to HPCE analysis of the linear ODN-CPG sample.

Therefore, examiner concludes that one of ordinary skill in the oligonucleotide solid-phase synthesis art would have been motivated to incorporate the detritylation process step of Horn et al. **WA** into the linear oligonucleotide synthesis of Horn et al. **UA** to produce a more rapid and consequently more efficient synthesis as a means of generally improving the synthesis of any linear oligonucleotide. Examiner also notes that applicant's original independent claims **1 and 21** were not limited to "linear oligonucleotides."

For these reasons the above grounds of rejection have been maintained.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. §1.136(a).

A shortened statutory period for response to this final action is set to expire THREE MONTHS from the date of this action. In the event a first response is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R. §1.136(a) will be calculated from the mailing date of the advisory action. In no event will the statutory period for response expire later than SIX MONTHS from the date of this final action.

Papers related to this application may be submitted to Group 1600 via facsimile transmission(FAX). The transmission of such papers must

conform with the notice published in the Official Gazette (1096 OG 30, November 15, 1989). The telephone numbers for the FAX machines operated by Group 1600 are **(703) 308-4556** and **703-305-3592**.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner L. E. Crane whose telephone number is 703-**308-4639**. The examiner can normally be reached between 9:30 AM and 5:00 PM, Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. James O. Wilson, can be reached at (703)-308-4624.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group 1600 receptionist whose telephone number is 703-**308-1235**.

LECrane:lec
01/28/03

James O. Wilson
Supervisory Patent Examiner
Technology Center 1600